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(54) **ANTI-INFLAMMATORY EYE DROPS**

(57) The present invention relates to an anti-inflammatory eye drop comprising a drug selectively inhibiting COX-2, selected from the group consisting of etodolac, N-(2-(cyclohexyloxy)-4-nitrophenyl) methanesulfonamide and meloxicam, which only slightly damages corneal epithelium and conjunctiva and which has an excellent anti-inflammatory effect.

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**Description****Technical Field**

5 **[0001]** The present invention relates to a therapeutic agent for treating inflammatory diseases of eyes in which prostaglandin serves as a mediator of inflammation and more specifically to an eye drop for preventing and treating anterior ophthalmic inflammatory diseases observed, for instance, after the operation of cataract.

**Background Art**

10 **[0002]** A non-steroid anti-inflammatory agent such as those containing sodium diclofenac, which shows its anti-inflammatory effect through the inhibition of the biosynthesis of prostaglandin as a mediator of the inflammation, is used not only for the treatment of inflammatory diseases by oral administration, but also for treating a variety of inflammatory diseases through local administration. In addition, it has also widely been used, in the form of an eye drop as a locally administered drug, for treating ophthalmic inflammatory diseases, in particular, anterior ophthalmic inflammatory symptoms after the operation of cataract and complications observed during and after the operation.

15 **[0003]** On the other hand, such a non-steroid anti-inflammatory eye drop is excellent in the anti-inflammatory action, but there has clinically been pointed out the occurrence of a side effect such as disorders of corneal epithelium at a frequency of about 1.6% (see the document attached to a medicine manufactured and sold by Wakamoto Pharmaceutical Co., Ltd., 1996). In other words, there has been desired for the development of a non-steroid anti-inflammatory eye drop, which is not accompanied by a high probability of causing disorders of corneal epithelium as a side effect.

20 **[0004]** As a result of the recent progress in research, there have been pointed out, as sites of action of the non-steroid anti-inflammatory agent, inhibition of two enzymes, i.e., cyclooxygenase-1 (hereinafter referred to as "COX-1") and cyclooxygenase-2 (hereinafter referred to as "COX-2"). It has been recognized that COX-1 serves to protect cells while COX-2 is an enzyme involved in inflammation. For this reason, there has been a desire for the development of an anti-inflammatory agent, which can selectively inhibit COX-2 and has a low probability of causing disorders of cells. The development of gastric ulcer as a side effect of a systemically administered anti-inflammatory agent has been studied in detail from such a standpoint discussed above. As a result, the roles of COX-1 and COX-2 have almost completely been elucidated and it has also been proved that the inhibition of COX-1 is involved in the development of gastric ulcer.

30 **[0005]** As has been described above, the mechanism of the action of these anti-inflammatory agents in the gastric ulcer has already been elucidated, but it has not yet been clear whether the disorders of the corneal epithelium are caused by the same mechanism of action as that of the gastric ulcer or not. There has thus been a desire for the development of a drug, which permits the distinct discrimination of the roles of COX-1 and COX-2 in disorders of the corneal epithelium and in ophthalmic inflammatory diseases. Masferrer, JL et al. (Surv. Ophthalmol., 1997, 41 (suppl. 2): S35-S40) reports that anterior ophthalmic inflammation can be suppressed by a COX-2 inhibitor, but this article does not include any disclosure concerning the disorders of corneal epithelium. Miyake, K. (Clinical Ophthalmologists' Reports, 1997, 51(11): 190-191) suggests that the use of a selective COX-2-inhibitor can relieve disorders of the corneal epithelium, but the article does not include any specific disclosure concerning means for solving the same.

**Disclosure of the Invention**

40 **[0006]** The inventors of this invention have used a drug, which selectively inhibits COX-2, among non-steroid anti-inflammatory drugs as an eye drop, have investigated the effect thereof on anterior ophthalmic inflammatory diseases and disorders of ophthalmic cells and thus have completed the present invention.

45 **[0007]** More specifically, the present invention relates to an anti-inflammatory eye drop containing, as an effective component, a drug having high COX-2 selectivity. The inventors of this invention have tried to inspect compounds having COX-1 and COX-2 selectivity for an anti-inflammatory effect in vivo and an ability of damaging cells in vitro and as a result, have found that an anti-inflammatory eye drop containing, as an effective component, a drug having high COX-2 selectivity is excellent in anti-inflammatory effect and in the alleviation of cell-damage. Accordingly, it is an object of the present invention to provide an anti-inflammatory agent, which can alleviate any damage of cells such as corneal epithelial cells and conjunctival cells and an eye drop, which does not cause severe disorders of the corneal epithelium.

**Brief Description of the Drawings**

55 **[0008]**

Fig. 1 is a graph showing the effect of etodolac on the PGE<sub>2</sub> content, which is increased by the anterior chamber puncture.

Fig. 2 is a graph showing the effect of etodolac on the protein content, which is increased by the anterior chamber puncture.

### Best Mode for Carrying Out the Invention

**[0009]** Preferred examples of non-steroid anti-inflammatory drugs having high COX-2 selectivity and used in the eye drop of the present invention are etodolac (1,8-diethyl-1,3,4,9-tetrahydropyrano [3,4-b] indole-1-acetic acid), N-(2-(cyclohexyloxy)-4-nitrophenyl) methanesulfonamide (NS-398) and meloxicam (4-hydroxy-2-methyl-N-(5-methyl-2-thiazolyl)-2H-1,2-benzothiazine-3-carboxamide 1,1-dioxide).

**[0010]** The eye drop of the present invention is desirably a sterilized pharmaceutical preparation, the preparations containing etodolac or NS-398 may comprise castor oil, sesame oil or other surfactants as a solubilizing agent and these effective components may be incorporated into ointments.

**[0011]** Meloxicam is also a drug having high COX-2 selectivity and is characterized in that it is highly water-soluble as compared with the foregoing drugs. Therefore, this drug can be used in an aqueous eye drop and may likewise be used in the form of an ointment.

**[0012]** The preferred concentration of such a drug used for treating inflammatory diseases ranges from 0.1% to 1% for meloxicam as an aqueous pharmaceutical preparation and 0.5% to 5% for an oily pharmaceutical preparation containing, for instance, etodolac or N-(2-(cyclohexyloxy)-4-nitrophenyl) methanesulfonamide. The effects of these drugs are confirmed by the inflammation model test.

**[0013]** In respect of the possibility of damaging cells, any drug having COX-2 selectivity shows low probability of damaging corneal epithelial cells and conjunctival cells. In particular, when exposing cells to the drugs for a long period of time, it has been clear that these drugs hardly cause disorders of the corneal epithelium.

**[0014]** As has been described above, the present invention has, for the first time, provided an eye drop, which hardly damages cells such as corneal epithelial cells or conjunctival cells, in particular, an eye drop containing a selective COX-2 inhibitor, which shows high anti-inflammatory effect without causing any severe disorders of the corneal epithelium.

**[0015]** The present invention will further be described, in more detail with reference to the following working Examples and Test Examples.

### Example 1

**[0016]**

Etodolac	5 g
Propyl p-Oxybenzoate	0.01 g
Methyl p-Oxybenzoate	0.05 g
Castor Oil	to 100 ml

**[0017]** To 80 ml of castor oil, there were added propyl p-oxybenzoate and methyl p-oxybenzoate to thus dissolve them in the castor oil, then castor oil was further added to give a total volume of 100 ml and the resulting solution was sterilized by filtration to give an eye drop according to the present invention.

### Example 2

**[0018]**

N-(2-(cyclohexyloxy)-4-nitrophenyl) methanesulfonamide	5 g
Propyl p-Oxybenzoate	0.01 g
Methyl p-Oxybenzoate	0.05 g
Castor Oil	to 100 ml

**[0019]** To 80 ml of castor oil, there were added N-(2-(cyclohexyloxy)-4-nitrophenyl) methanesulfonamide, propyl p-oxybenzoate and methyl p-oxybenzoate to thus dissolve them in the castor oil, then castor oil was further added to give a total volume of 100 ml and the resulting solution was sterilized by filtration to give an eye drop according to the present invention.

### Example 3

**[0020]**

Meloxicam	0.5 g
Tween-80	0.5 g
Methyl Cellulose	0.5 g
Boric Acid	0.1 g
EDTA	0.005 g
Benzalkonium Chloride	0.005 g
0.1N HCl/0.1N NaOH (an amount required for adjusting the pH to 7.2)	
Purified Water	to 100 ml

**[0021]** To 80 ml of purified water, there were added meloxicam, Tween-80 (Polysorbate-80), methyl cellulose, boric acid, EDTA and benzalkonium chloride to thus dissolve these components in water. The pH value of the resulting solution was adjusted to 7.2 by the addition of 0.1N HCl or 0.1N NaOH and purified water was further added to the solution to give a total volume of 100 ml. The resulting solution was sterilized by filtration to give an eye drop of the present invention.

**[0022]** The method of using the eye drop of the present invention and the volume thereof to be administered may vary depending on, for instance, the symptoms of the patients and age thereof, but the eye drop is in general dropped in the eyes in a dose of 1 to 2 drops over one to 6 times per day.

### Test Example 1: Effect of Eye Drop on Inflammation in Anterior of White Rabbit

**[0023]** In this Test Example, there were used Japanese white rabbits (available from Japan Medical Animal Source Laboratory) each having a body weight ranging from 1.8 to 2.4 kg and they were divided into groups each consisting of 6 to 7 animals.

**[0024]** After administration of 250 U/kg of heparin (available from Takeda Chemical Industries, Ltd.) to these rabbits through the ear veins thereof, a test substance was dropped in the both eyes in an amount of about 60  $\mu$ l. After 45 minutes from the dropping of the substance in the eyes, these animals were locally anesthetized with benoxil eye drop (available from Santen Pharmaceutical Co., Ltd.) and then all of the anterior chamber fluid was collected using a 27G injection needle. This anterior chamber fluid was defined, to be a primary aqueous humor. After 90 minutes from the collection of the aqueous humor, the rabbits were killed by anesthetization with an excess of pentobarbital sodium and the aqueous humor was again collected. This anterior chamber fluid was defined to be a secondary aqueous humor.

**[0025]** The concentration of prostaglandin  $E_2$  (hereinafter referred to as "PGE<sub>2</sub>") and the amount of proteins present in the collected secondary aqueous humor were determined and they were used as indications of inflammation. After pre-treatment of 100  $\mu$ l of the aqueous humor using a Bond Elute C<sub>18</sub> column, the concentration of PGE<sub>2</sub> was determined using Biotrack PGE<sub>2</sub> EIA system (available from Amersham Company). On the other hand, the amount of proteins was determined according to the Lowry method using bovine serum albumin (available from Nakarai Co., Ltd.) as a reference protein.

**[0026]** Etodolac as a test substance was dissolved in castor oil specified in the pharmacopoeia to concentrations of 0.5% and 5%. Meloxicam was diluted with physiological saline prior to use.

**[0027]** As will be seen from the data shown in Figs. 1 and 2, etodolac inhibited the increase in the PGE<sub>2</sub> content and the protein content present in the aqueous humor, due to the anterior chamber puncture.

**[0028]** In addition, meloxicam inhibited increases of the protein and PGE<sub>2</sub> contents in a concentration ranging from 0.1 to 1.0% and the inhibitory effect thereof was found to be conspicuous as compared with other drugs.

Table 1

Effect of Meloxicam on the Increase of Protein Content and PGE <sub>2</sub> Content after the Anterior Chamber Puncture				
	Control	Meloxicam		
		0.1%	0.5%	1.0%
Protein Content (mg/ml) Inhibition Rate	22.9±1.0	16.2±1.9** 29.3%	11.0±1.5*** 52.2%	12.0±2.1*** 47.6%
PGE <sub>2</sub> Content (pg/ml) Inhibition Rate	2717.4±506.8	680.6±239.9* 75.0%	253.5±67.0*** 90.7%	158.3±30.0*** 94.2%
Each value in this Table is an average of 11 to 12 eyes. *, **, ***: P<0.05, 0.01, 0.001: Dunnett's Test				

**Test Example 2: Influence of Drugs on Uveitis Induced by LPS**

**[0029]** In this Test Example, groups of Japanese white male rabbits each having a body weight ranging from 1.7 to 2.4 kg were used, wherein each group consisted of 12 to 16 eyes.

**[0030]** The lipopolysaccharide (LPS) (055: B5 type, available from Sigma Company) derived from E. coli was administered to these rabbits through the auricular veins in a dose of 1.25µg/kg to thus induce uveitis. After 4 hours from the administration of LPS, the rabbits were killed by anesthetization with an excess of pentobarbital sodium (available from Tokyo Chemical Industry Co., Ltd.) and the anterior chamber fluid, was collected.

**[0031]** The PGE<sub>2</sub> concentration and the amount of proteins present in the collected aqueous humor were determined according to the same methods used in Example 1.

**[0032]** In this respect, one hour after administering LPS each test substance was dropped in both eyes of these animals at a dose of about 60 µL/eye.

**[0033]** NS-398 as a test substance was dissolved or suspended in castor oil specified in the pharmacopoeia to concentrations of 0.5% and 5%. Meloxicam was diluted with physiological saline prior to use.

**[0034]** As will be clear from the data shown in the following Table 2, NS-398 considerably inhibited the increase in the amount of PGE<sub>2</sub>. In addition, as will be clear from the data listed in the following Table 3, meloxicam inhibited increases of the protein and PGE<sub>2</sub> contents in a concentration ranging from 0.1 to 1.0% and thus, it was found to have an excellent anti-inflammatory effect.

Table 2

Effect of NS-398 on Uveitis Induced by LPS			
	Control	NS-398	
		0.5%	5.0%
PGE <sub>2</sub> Content (pg/ml)	1336.3±380.1	455.3±118.6##	542.8±79.8 <sup>#</sup>
Inhibition Rate		65.9%	59.4%
Each value in this Table is an average of 12 to 16 eyes. #, ##: P<0.05, 0.01: Dunnett's Test			

Table 3

Effect of Meloxicam on Uveitis Induced by LPS				
	Control	Meloxicam		
		0.1%	0.5%	1.0%
Protein Content (mg/ml)	24.2±0.3	16.0±1.9**	14.3±2.6**	12.2±1.8***
Inhibition Rate		33.8%	40.8%	49.4%
PGE <sub>2</sub> Content (pg/ml)	2500.4±647.3	1102.8±139.4	780.9±189.4**	922.8±291.2**
Inhibition Rate		55.9%	68.8%	63.1%
Each value in this Table is an average of 12 eyes. **, ***: P<0.01, 0.001: Dunnett's Test				

### Test Example 3: Influence of Cyclooxygenase-Inhibitory Agent on Corneal Epithelial Cells and Conjunctival Cells

#### [0035]

**Cells Used:** There were used SV40 immortalized human corneal epithelial cell strains (Araki-Sasaki et al., IOVS, 1995, 36:614-621) as the corneal cells and Chang human conjunctival cell strains (ATCC CCL-20.2) as the conjunctival cells.

**Test Method:** These cells were cultured in 96-well plate to a confluent of 30 to 50% and then each test substance stepwise diluted with the culture medium was added to the foregoing culture medium to thus carry out cultivation at 37°C. After completion of the cultivation, the plate was washed with PBS(-) and the number of residual cells was determined in terms of the  $\beta$ -hexosaminidase activity.

**Method for Evaluating Toxicity:** A group treated with only the solvent, which was used for dissolving the test substance was defined to be a negative control, the viable cell number observed for the control was assumed to be 100%, a dose-correlation curve was prepared on the basis of the viable cell number observed at each concentration of test substance and the concentration of each test substance required for achieving the survival rate of 50% (EC50) was determined from the curve and it was defined to be the toxicity value thereof.

**[0036]** As will be clear from the data listed in the following Table 4, etodolac which has relatively high selectivity for COX-2 shows low probability of damaging cells such as corneal epithelial cells and conjunctival cells, as compared with compounds which have low selectivity for COX-1 and COX-2, such as indometacin or diclofenac sodium. This would lead to the development of a non-steroid anti-inflammatory agent, which hardly causes disorders of cornea.

**[0037]** In addition, the data shown in the following Table 5 indicate that meloxicam only quite weakly damages the corneal epithelial cells and conjunctival cells.

Table 4

Influence of Non-Steroid Anti-inflammatory Agents on Corneal Epithelial and Conjunctival Cells		
	Corneal Epithelial Cells EC50 (mM)	Conjunctival Cells EC50 (mM)
Diclofenac Sodium	0.30	0.13
Indometacin	0.57	0.37
Etodolac	1.00	0.80
Note: The cells were exposed to each test substance for 24 hours.		

Table 5

Influence of Non-Steroid Anti-inflammatory Agents on Corneal Epithelial and Conjunctival Cells				
	Corneal Epithelial Cells EC50 (mM)		Conjunctival Cells EC50 (mM)	
Test Substance-Exposure Time (hr)	24	48	24	48
Meloxicam	1.0<	0.84	0.97	0.38
Diclofenac Sodium	0.63	0.23	0.23	0.22

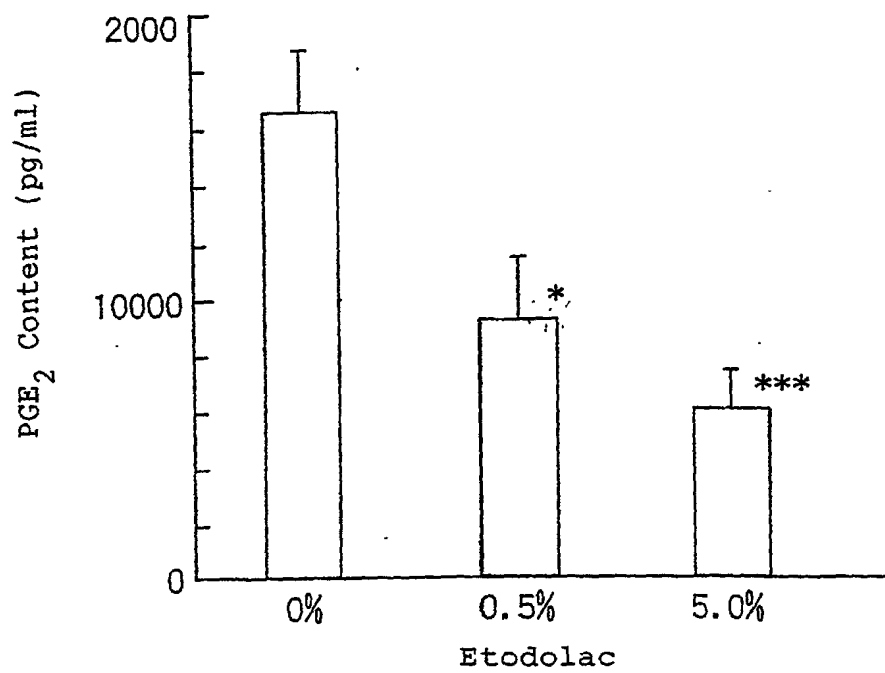
### Industrial Applicability

**[0038]** The drug of the present invention, which can selectively inhibit COX-2, can be used in the form of an eye drop, which quite slightly damages the cornea and can show conspicuous effect in the treatment of ophthalmic inflammatory diseases. In particular, meloxicam has an excellent effect even when exposing it to the corneal epithelial cells over a long period of time as compared with the conjunctival cells.

### Claims

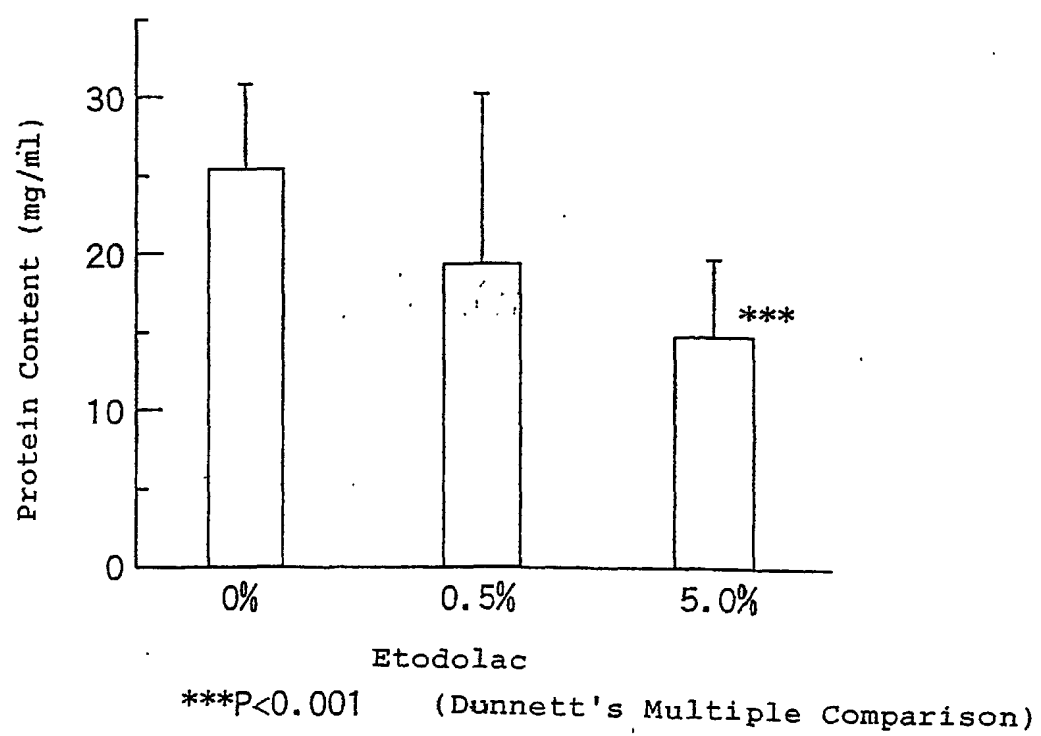
1. An eye drop for treating ophthalmic inflammatory diseases, comprising, as an effective component, a drug selectively inhibiting cyclooxygenase-2.
2. The eye drop of claim 1 wherein the drug selectively inhibiting cyclooxygenase-2 is etodolac.
3. The eye drop of claim 2 wherein the concentration of etodolac ranges from 0.5 to 5%.
4. The eye drop of claim 1 wherein the drug selectively inhibiting cyclooxygenase-2 is N-(2-(cyclohexyloxy)-4-nitrophenyl) methanesulfonamide.
5. The eye drop of claim 4 wherein the concentration of N-(2-(cyclohexyloxy)-4-nitrophenyl) methanesulfonamide ranges from 0.5 to 5%.
6. The eye drop of claim 1 wherein the drug selectively inhibiting cyclooxygenase-2 is meloxicam.
7. The eye drop of claim 6 wherein the concentration of meloxicam ranges from 0.1 to 1%.

FIG. 1



\*P<0.05, \*\*\*P<0.001 (Dunnett's Multiple Comparison)

FIG. 2



## INTERNATIONAL SEARCH REPORT

International application No.

PCT/JP99/02522

<b>A. CLASSIFICATION OF SUBJECT MATTER</b> Int.Cl <sup>6</sup> A61K45/00, A61K31/18, A61K405, A61K31/54 According to International Patent Classification (IPC) or to both national classification and IPC		
<b>B. FIELDS SEARCHED</b> Minimum documentation searched (classification system followed by classification symbols) Int.Cl <sup>6</sup> A61K45/00, A61K31/18, A61K405, A61K31/54 Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) CAPLUS (STN), MEDLINE (STN)		
<b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	MASFERRER J. L., et al., "Cyclooxygenase-2 inhibitors: a new approach to the therapy of ocular inflammation." SURVEY OF OPHTHALMOLOGY, 1997, Vol. 41, Suppl. 2 S35-40 & Database Medline on STN, US National Library of Medicine, (Bethesda, MD, USA), No.97298858	1-7
Y	WO, 95/18604, A1 (Ciba-Geigy A.-G.), 13 July, 1995 (13. 07. 95) whole document, (No family)	1-7
<input type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/> See patent family annex.		
* Special categories of cited documents: "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier document but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family		
Date of the actual completion of the international search 9 August, 1999 (09. 08. 99)		Date of mailing of the international search report 17 August, 1999 (17. 08. 99)
Name and mailing address of the ISA/ Japanese Patent Office		Authorized officer
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